

ment can include a fan for directing an air stream at the thermally conductive exterior wall of the amplification device. Alternatively, the instrument can include a heat sink for making reversible contact with the thermally conductive exterior wall of the amplification device. The instrument can also be equipped with an electrical connector for contacting a Peltier circuit on the thermally conductive exterior wall of the amplification device. An electrical connector provided with the instrument can also be used for contacting a fluid detection sensor in the amplification device.

**[0040]** According to exemplary embodiments, a method is also provided of nucleic acid amplification for producing an amplicon in a single-use device. The method comprises the steps of introducing a nucleic acid sample into an amplification chamber through a sample entry orifice, sealing the orifice, transferring a fluid from a fluid pouch through a reversibly sealable ingress to the amplification chamber, sealing the ingress and an egress of the chamber, mixing the fluid with the sample to form a mixture comprising nucleic acid, buffer, a polymerase and one or more primers, cycling the temperature of the chamber between a first and second temperature for a predetermined time and for a predetermined number of cycles to form an amplicon, opening the ingress and egress of the chamber, and applying a pneumatic force to the ingress to move the amplicon from the chamber through the egress.

**[0041]** Yet another method according to an alternative exemplary embodiment comprises the steps of introducing a nucleic acid sample into an amplification chamber through a sample entry orifice, sealing the orifice, transferring a fluid from a fluid pouch through a reversibly sealable ingress to the amplification chamber, sealing the ingress and an egress of the chamber, mixing the fluid with the sample to form a mixture comprising nucleic acid, buffer, a polymerase and one or more primers, increasing the temperature of the chamber to an isothermal amplification temperature for a predetermined time to form an amplicon, opening the ingress and egress of the chamber, and applying a pneumatic force to the ingress to move the amplicon from the chamber through the egress.

**[0042]** More particularly, according to a first aspect of the present invention, a single-use nucleic acid amplification device for producing an amplicon includes a housing and an amplification chamber. The amplification chamber includes an ingress with a first reversible seal, an egress with a second reversible seal, a sealable sample entry orifice, and a first wall forming a portion of the amplification chamber. The first wall comprises a thermally conductive material and includes a first surface and a second surface. The second surface includes a heating circuit and a temperature sensor. The sample entry orifice is configured to permit a sample of nucleic acid to enter the amplification chamber. The ingress is connected to a first conduit along with a pump and a reservoir. The egress is connected to a second conduit permitting egress of the amplicon from the amplification chamber.

**[0043]** According to the first aspect, the pump can comprise a flexible diaphragm or the like. For example, the flexible diaphragm can be capable of engaging and being actuated by a plunger on an instrument with which the amplification device is capable of mating. Alternatively, the flexible diaphragm is capable of manual actuation. The pump can comprise, for example, a pneumatic pump or other like device or mechanism. The reservoir can comprise, for

example, a fluid pouch or the like. The fluid pouch can include a fluid for performing nucleic acid amplification. The fluid pouch can include a fluid for performing a nucleic acid amplification and one or more reagents. Each reagent can comprise at least one of deionized water, a buffer material, dNTPs, one or more primers, and a polymerase. The reservoir can comprise a flexible diaphragm. The flexible diaphragm can be capable of engaging and being actuated by a plunger on an instrument with which the amplification device is capable of mating. Alternatively, the flexible diaphragm can be capable of manual actuation.

**[0044]** According to the first aspect, the first wall can comprise silicon or other like material. For example, the silicon can comprise about 30 to about 50 percent of the first surface area of the amplification chamber. The amplification chamber can comprise a second wall made of a plastic material. For example, the second wall can comprise a wall thickness in the range of about 0.2 mm to about 5 mm, and the second wall can include one or more additional rib supports. The internal volume of the amplification chamber can be in the range of about 5  $\mu$ L to about 50  $\mu$ L. The amplification chamber surface to an amplification chamber volume ratio can be in the range of about 50 to about 200 square mm for the amplification chamber surface and to about 5 to about 30 cubic mm for the amplification chamber volume. The internal shape of the amplification chamber can comprise one of a substantially rectangular structure, a substantially rectangular shape with rounded corners, a cylinder, a cylindrical structure with a substantially oval cross-section, and other like structures or configurations. The second surface of the first wall can comprise a heating circuit. The heating circuit can comprise a resistive electrical path fabricated on the second surface with a first and second connecting pad for contacting an external circuit for providing current flow through the path. The second surface of the first wall can comprise a temperature sensor. The temperature sensor can comprise a thermistor or a thermocouple fabricated on the second surface with a first and second connecting pad for contacting an external circuit for connecting to the one of the thermistor and the thermocouple.

**[0045]** According to the first aspect, the sample entry orifice can be capable of mating with a sample introduction element. The sample introduction element can comprise a wand. The wand can comprise a first end with an absorbent pad capable of collecting and retaining a nucleic acid sample. The wand can also comprise a second end forming a handle. The first end can be capable of passing through the sample entry orifice into the amplification chamber. The wand can include an engaging structure between the first and second ends for engaging and sealing the wand in the sample entry orifice. For example, the engaging structure can comprise a male screw structure on the wand and a female screw structure on the sample entry orifice. Alternatively, the engaging structure can comprise a male collar locking structure on the wand and a female collar locking structure on the sample entry orifice. The amplification chamber can contain, for example, a polymerase and dNTPs. Additionally or alternatively, the amplification chamber can contain one or more primers. The amplification chamber can contain a buffer. The amplification chamber can comprise, for example, a sugar glass coating on at least a portion of the first surface of the first wall. The sugar glass coating can comprise a reagent or the like. The reagent can comprise at least one of a buffer, a dye, one or more primers, and a